Sensorimotor network hypersynchrony as an Endophenotype in Families with Genetic Generalised Epilepsy: a resting state fMRI study

Chayanin Tangwiriyasakul [1*], Suejen Perani [1,2*], Eugenio Abela [1], David W. Carmichael [2,3**], Mark P. Richardson [1,4**]

1 Department of Basic and Clinical Neuroscience, Institute of Psychiatry Psychology and Neuroscience, King's College London, London, UK

2 UCL Great Ormond Street Institute of Child Health, UCL, London, UK

3 School of Imaging and Bioengineering, faculty of Life Sciences and Medicine, King's College London, London, UK

4 Centre for Epilepsy, King's College Hospital, London, UK

* These authors share first authorship.

** These authors share senior authorship.

Corresponding author:

Dr. C. Tangwiriyasakul (PhD)
King's College London, Institute of Psychiatry Psychology & Neuroscience
5 Cutcombe Road, London SE5 9RX, UK
Tel: + 44(0)20 7848 5162
Email: chayanin.tangwiriyasakul@kcl.ac.uk

Key words: Genetic Generalised Epilepsy (GGE), Endophenotype pf GGE, BOLD-fMRI

Number of pages: 12 pages

Number of words (summary): 198 words

Number of words (main text): 1976 words

Number of words (2nd short summary): 98 words

Number of figures: 2 figure (a color figure in the mainbody and a greyscale figure in the supplementary)

Number of tables: 4 tables (one in the main text, three in the supplementary)

Number of references: 21

Summary

Recent evidence suggests that three specific brain networks show state-dependent levels of synchronisation before, during and after episodes of generalised spike-wave discharges (GSW) in patients with Genetic Generalised Epilepsy (GGE). Here, we investigate whether synchronisation in these networks differs between patients with GGE (n = 13), their unaffected first-degree relatives (n = 17), and healthy controls (n = 18). All subjects underwent two 10-minute simultaneous EEG-fMRI recordings without GSW. Whole-brain data were divided into 90 regions, and BOLD-phase-synchrony in a 0.04-0.07Hz band was estimated between all pairs of regions. Three networks were defined: (1) the network with highest synchrony during GSW events, (2) a sensorimotor network, (3) an occipital network. Average synchrony (mean node degree) was inferred across each network over time. Notably, synchrony was significantly higher in the sensorimotor network in patients and in unaffected relatives, compared to controls. There was a trend towards higher synchrony in the GSW network in patients and in unaffected relatives. There was no difference between groups for the occipital network. Our findings provide evidence that elevated fMRI-BOLD synchrony in a sensorimotor network is a state-independent endophenotype of GGE, present in patients in the absence of GSW, and present in unaffected relatives.

1. Introduction

There is strong evidence for the heritability of Genetic (or Idiopathic) Generalised Epilepsy (GGE) [1]. An important concept emerging in studies of disease inheritance is endophenotype [2], a heritable trait with a simpler genetic basis than the full disorder, which may be present in family members who do not have the disease. There is increasing interest in identifying endophenotypes in epilepsy.

A few studies have already demonstrated that GGE may have a distinct endophenotype. For example, we found enhanced EEG network synchrony in patients with GGE and unaffected first-degree relatives [3]. Using fMRI, hyperconnectivity between a network engaged in a cognitive task and the sensorimotor network was found in both patients with GGE and first degree relatives [4; 5]. An endophenotype is a heritable trait which is a component of a disorder or associated with high liability to develop the disorder. An endophenotype may be present in family members who do not have the disease, hence increasing the power of genetic studies. This concept has allowed the genetic dissection of complex disorders such as Rolandic epilepsy [6; 7].

In a recent study, we found that synchrony in specific networks, observed with BOLD fMRI and simultaneous EEG, varies dynamically around the time of generalized spike wave (GSW) events observed in EEG [8], apparently anticipating the onset of GSW by several seconds. We also noted that, remote from GSW events, there was evidence that network synchrony was higher in patients than healthy controls in a sensorimotor network. Here, we examine whether this elevated synchrony is also present in unaffected first-degree relatives of patients with GGE.

2. Methods

Participants

We have previously acquired and published EEG-fMRI data from 21 patients diagnosed with Juvenile Myoclonic Epilepsy (JME) or Generalised Tonic-Clonic Seizures Only (GTCSO) [8]; here we include the 13 in whom fMRI runs were entirely free of GSW (mean age 20.5 ± SD 6.6 years), in addition to 18 healthy controls reported in the same prior study (mean age 23.9 ± SD 3.8 years), and 17 unaffected first-degree relatives collected during the same period of time, but not previously reported (mean age 39.4 ± SD 14.2 years, see supplementary Table A1). Note that none of the relatives were related to any of the patients in this study. Patients were recruited through clinics across south east London. Relatives were recruited via patients with JME or GTCSO attending these clinics. Participants were excluded if they had any neurological diagnoses other than epilepsy or history of drug or alcohol misuse. Healthy controls and first degree relatives did not have any history of seizures or epilepsy. This study was approved by the Riverside Research Ethics Committee (REC approval number 12/LO/2006 and REC approval number 11/LO/1421) and all participants gave written informed consent according to the Declaration of Helsinki (2013).

Data acquisition and preprocessing

Participants underwent two runs of simultaneous resting-state EEG-fMRI on a 3T MR750 scanner (GE Healthcare, Milwaukee, Wisconsin) to acquire 300 BOLD echo-planar images per run (3.3mm isotropic voxels, FOV 211mm, TR 2.160s, TE 25ms, flip angle 75 degrees, 36 slices, thickness 2.5 mm). During scanning, all subjects were asked to rest with their eyes closed. EEG data were acquired at 5000 Hz with an MRI-compatible EEG cap containing 63 Ag/AgCl electrodes referenced to FCz (Brain Products GmbH, Munich, Germany). Impedances were

kept under 10 kOhm. MR gradient and pulse-related artefacts were removed off-line from the EEG recorded inside the MRI using template artefact subtraction (Brain Analyzer, Brain Products) [9; 10]. To preprocess fMRI data, we used SPM8 (r6313, www.fil.ion.ucl.ac.uk/spm) running on MATLAB (R2017b) and the FIACH package (www.homepages.ucl.ac.uk/~ucjttie/FIACH.html) to correct for physiological artefacts in the BOLD time series [11]. Next, we normalized the corrected data into the standard MNI space. Finally, all images were spatially smoothed using Gaussian kernel of 8 mm full-width at half maximum.

fMRI Data analysis

Full details of data analysis were reported in our previous study [8]. In summary, BOLD signals were first bandpass filtered between 0.04-0.07 Hz [12]. We then parcellated the brain into 90 regions using automatic anatomical labelling (AAL) [13]. The first principal component of voxel timeseries was used to represent each brain region [14]. Next, the Hilbert transform was applied to estimate instantaneous phase of the first principal component in each region. Subsequently, we estimated a time-varying phase difference matrix by subtracting the phase angle between pairs of regions, resulting in a 90x90x285 adjacency matrix for each fMRI run Note that for each run the first 10 TR and the last 5 TR from 300 TRs were excluded because fMRI noise seen in the EEG. We binarised these matrices using a threshold of pi/6 [8; 15]. Tensor decomposition was applied to the series of adjacency matrices for each run to try to reduce the number of spurious network connections [8].

EEG Data analysis

We used alpha power estimated from O1, O2, and Oz to monitor the level of vigilance of each subject in each run, in order to take account of likely change in vigilance over the duration of each scan run [16]. To avoid fMRI noise in the EEG, that would prevent estimation of alpha power, we excluded the first 21.6 seconds [10TR] and the last 10.8 second [5TR] of each EEG. Each EEG was bandpass filtered between 8-12 Hz. Then we estimated the alpha power over each period consecutive period of 10 seconds. To avoid inter-subject variability, we normalised the alpha power by dividing by the broadband EEG power (1-40 Hz). For each run, we estimated the slope of normalised alpha power, representing a change in the level of vigilance. This slope was later used as a covariate.

Estimation of average network synchrony

In our previous study, we examined time-varying network synchrony around the time of occurrence of GSW discharges, and around random events, in the same subjects [8]. We observed three canonical networks in these data: (1) a network prominent during GSW in patients (GSW network), (2) a network prominent prior to GSW in patients (sensorimotor network), and (3) a network prominent in healthy controls at the time of random events (occipital network) (see Table-A2 for a list of brain regions included in each network). We estimated network synchrony in these three canonical networks. As in our previous study, we used mean degree to measure network synchrony [8]. For each run, we took the phase synchrony matrices [90x90x285] obtained from the previous step, and then subsampled by including only the regions within each network, resulting in a matrices with $p \times p \times 285$ elements, where *p* is the number of regions in the network. At each TR, we estimated mean degree, which is the average of all elements in the $p \times p$ matrix. This step was repeated for

each TR and averaged over all TRs in each run. Finally, we estimated normalised mean degree for each subject, which is the mean degree of each network divided by the mean degree over the entire brain (where $p = 90$).

Statistical analyses

Since the data in this study were non-normally distributed, as determined by one-sample Kolmogorov-Smirnov test, non-parametric methods were chosen. We first ran a rank analysis of covariance (Quade's test) to examine mean degree across the three groups [17], where age and level of vigilance were used as covariates. A Mann-Whitney test was used to compare between pairs of groups. We considered the results to be significant if $p<0.05$ after Bonferroni correction for three group comparisons.

3. Results

Patients and first degree relatives had significantly higher network synchrony (mean degree) in the sensorimotor network than in the control group, after adjustment for age and level of vigilance and Bonferroni correction (Figure 1 and Table 1). There was a nonsignificant trend towards higher network synchrony in patients and first degree relatives in the GSW network. There were no differences between groups in the occipital network.

4. Discussion

In this study, we found that mean degree, a measure reflecting the average level of BOLD signal phase synchronisation, was significantly higher in a sensorimotor network in patients with GGE and in relatives of patients with GGE than in healthy control subjects. The data were obtained with simultaneous EEG and were free from episodes of GSW, suggesting that this phenomenon is independent of seizures or epilepsy, and may represent an inherited endophenotype of GGE. There was also a trend that mean degree was higher in the interictal state in patients, and in relatives, in the network that becomes prominently synchronised during GSW.

Network connectivity in GGE: state versus trait

In our previous study [8], we found that phase synchronisation of BOLD signals in canonical brain networks varied over time, in particular showing differences in epochs around GSW events compared to epochs without. This finding suggests that brain network synchronisation may vary over seconds or longer prior to GSW onset on EEG, and may reflect the mechanisms responsible for the transition from normal brain activity to GSW. In the study reported here, we found that brain network synchrony was abnormally elevated in GGE patients remote from GSW events as well as in 1st degree relatives, suggesting this phenomenon is an invariant trait. In support of this suggestion, several other studies using various data modalities, including diffusion tensor imaging, functional MRI, and transcranial magnetic brain stimulation, have reported hyper-connectivity in sensorimotor related areas of patients with GGE [4; 18; 19].

Network connectivity in relatives of patients with GGE: endophenotype

In a previous study using EEG [3], we studied features of functional networks. We found, exclusively in the low-alpha 6-9Hz range, that clustering coefficient and the variance of mean degree differed between GGE patients and healthy controls, and also differed between relatives of GGE patients and healthy controls. These measures were global statistics of a whole-brain network and we did not attempt to examine specific subnetworks, such as a sensorimotor network. In subsequent theoretical work, we showed that the connectivity features of these networks specifically predispose it to ictal onset [20].

A previous study using fMRI in JME patients and their first degree relatives showed that connectivity between the network involved in a working memory task carried out during scanning, and a sensorimotor network, was increased both in patients and unaffected relatives [5]. Our study here extends these findings to show that excessive synchrony within the sensorimotor network itself is observable at rest. Although the relationship between observation of sensorimotor network hypersynchrony and the mechanism of GSW onset cannot be inferred from our data, we speculate that the endophenotype of sensorimotor hypersynchronisation plays a role in facilitating the engagement of large-scale brain circuits in GSW driven from localised nodes such as the precuneus [21].

Strengths and weaknesses of our study

Our findings of elevated synchronisation in brain networks at rest, without GSW, could only be made because we had simultaneous EEG. For obvious reasons, it would be impossible to say that the network phenomena we observed are state-independent unless we could

exclude the occurrence of GSW events during the fMRI scans. Our subject groups differ in age distribution, but our robust methodology (non-parametric statistics with inclusion of age and level of vigilance as covariates) allowed us to take an optimal approach despite this limitation. Furthermore, in post-hoc analyses using Mann-Whitney U test, we showed that there was no effect in patients or relative groups of gender, of GGE syndrome (i.e. effects were similar in male and female and in the JME and GTCSO groups), or of photosensitivity, see details in the supplementary Table A3.

Conclusion and future work

We found here evidence that fMRI BOLD hypersynchrony in a sensorimotor network is an endophenotype of GGE, present in patients and unaffected relatives. Future work should seek to understand the mechanisms and genetic underpinnings of this observation. The sensorimotor system is amenable to manipulation by techniques such as non-invasive brain stimulation (e.g. using TMS). This might allow the clinical relevance of the sensorimotor hypersynchrony to be tested in the future.

Acknowledgements

We thank all participants and patients for generous involvement in this study. We thank radiography staff at KCL for excellent technical support in data acquisition. The study was funded by Medical Research Council (MR/K013998/1).

Disclosure

The authors have no conflicts of interest to report. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm this report is consistent with those guidelines.

References

[1] Gallentine WB, Mikati MA. Genetic generalized epilepsies. *J Clin Neurophysiol* 2012;29:408-419.

[2] Gottesman, II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 2003;160:636-645.

[3] Chowdhury FA, Woldman W, FitzGerald TH, et al. Revealing a brain network endophenotype in families with idiopathic generalised epilepsy. *PLoS One* 2014;9:e110136.

[4] Vollmar C, O'Muircheartaigh J, Barker GJ, et al. Motor system hyperconnectivity in juvenile myoclonic epilepsy: a cognitive functional magnetic resonance imaging study. *Brain* 2011;134:1710-1719.

[5] Wandschneider B, Centeno M, Vollmar C, et al. Motor co-activation in siblings of patients with juvenile myoclonic epilepsy: an imaging endophenotype? *Brain* 2014;137:2469-2479.

[6] Addis L, Chiang T, Clarke T, et al. Evidence for linkage of migraine in Rolandic epilepsy to known 1q23 FHM2 and novel 17q22 genetic loci. *Genes Brain Behav* 2014;13:333-340.

[7] Smith AB, Kavros PM, Clarke T, et al. A neurocognitive endophenotype associated with rolandic epilepsy. *Epilepsia* 2012;53:705-711.

[8] Tangwiriyasakul C, Perani S, Centeno M, et al. Dynamic brain network states in human generalized spike-wave discharges. *Brain* 2018.

[9] Allen PJ, Polizzi G, Krakow K, et al. Identification of EEG events in the MR scanner: the problem of pulse artifact and a method for its subtraction. *Neuroimage* 1998;8:229-239.

[10] Allen PJ, Josephs O, Turner R. A method for removing imaging artifact from continuous EEG recorded during functional MRI. *Neuroimage* 2000;12:230-239.

[11] Tierney TM, Weiss-Croft LJ, Centeno M, et al. FIACH: A biophysical model for automatic retrospective noise control in fMRI. *Neuroimage* 2016;124:1009-1020.

[12] Glerean E, Salmi J, Lahnakoski JM, et al. Functional magnetic resonance imaging phase synchronization as a measure of dynamic functional connectivity. *Brain Connect* 2012;2:91-101.

[13] Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002;15:273-289.

[14] Friston KJ, Rotshtein P, Geng JJ, et al. A critique of functional localisers. *Neuroimage* 2006;30:1077-1087.

[15] Ponce-Alvarez A, Deco G, Hagmann P, et al. Resting-state temporal synchronization networks emerge from connectivity topology and heterogeneity. *PLoS Comput Biol* 2015;11:e1004100.

[16] Tagliazucchi E, von Wegner F, Morzelewski A, et al. Dynamic BOLD functional connectivity in humans and its electrophysiological correlates. *Front Hum Neurosci* 2012;6:339.

[17] Quade D. Rank Analysis of Covariance. *Journal of the American Statistical Association* 1967;62:1187-&.

[18] Caeyenberghs K, Powell HW, Thomas RH, et al. Hyperconnectivity in juvenile myoclonic epilepsy: a network analysis. *Neuroimage Clin* 2015;7:98-104.

[19] Badawy RA, Macdonell RA, Berkovic SF, et al. Predicting seizure control: cortical excitability and antiepileptic medication. *Ann Neurol* 2010;67:64-73.

[20] Petkov G, Goodfellow M, Richardson MP, et al. A critical role for network structure in seizure onset: a computational modeling approach. *Front Neurol* 2014;5:261.

[21] Vaudano AE, Laufs H, Kiebel SJ, et al. Causal Hierarchy within the Thalamo-Cortical Network in Spike and Wave Discharges. *PLoS One* 2009;4.

Figure

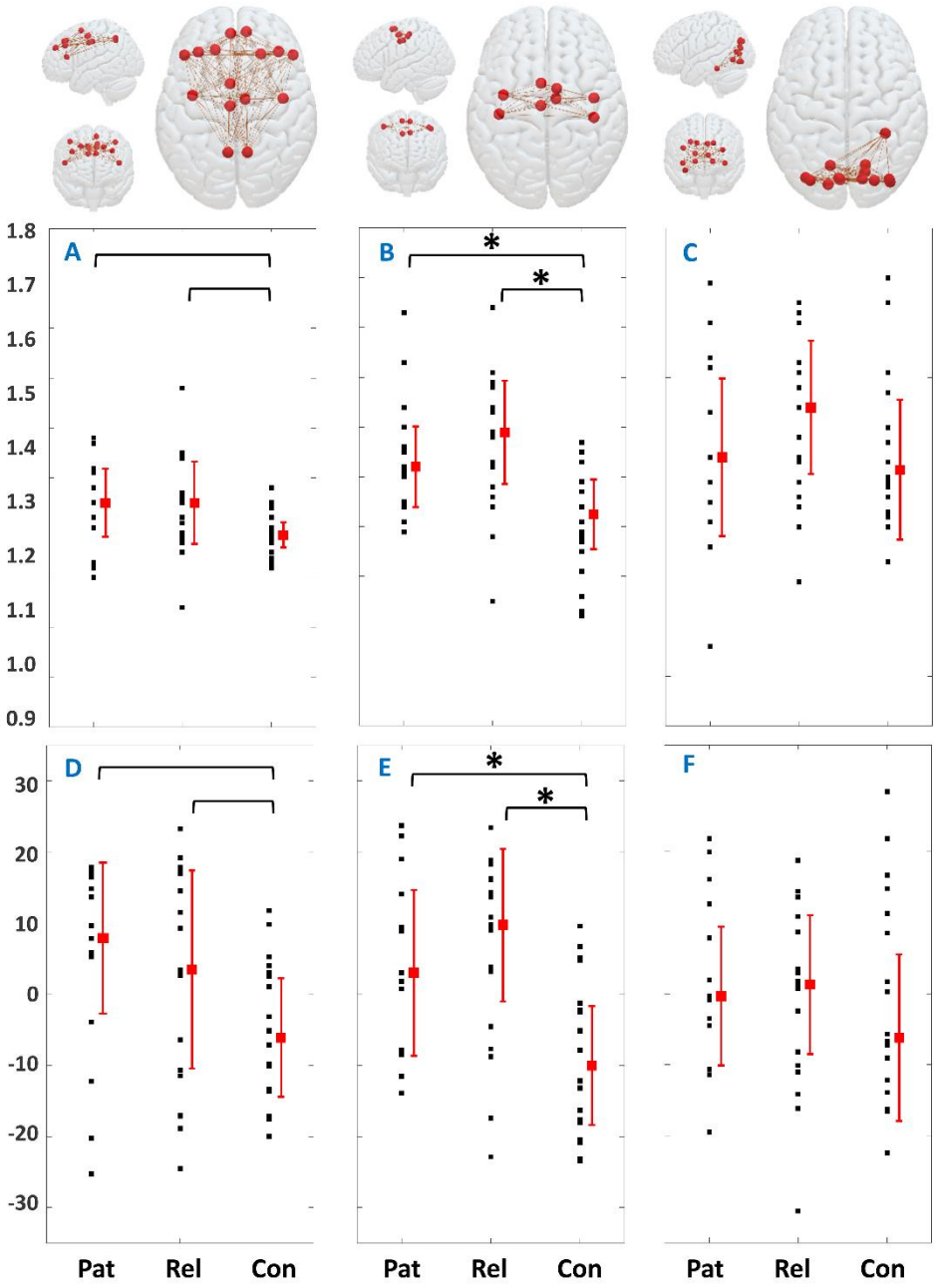

Figure 1: Average level of fMRI BOLD phase synchrony in the three canonical networks. (A) and (D) show GSW network; (B) and (E) sensorimotor network; and (C) and (F) occipital network. The top row shows cartoons of the networks involved. (A), (B) and (C) show mean degree uncorrected for age. (D), (E) and (F) show mean degree rank (centred on zero) adjusted for age and level of vigilance using Quade's ANOVA. Bracketed comparisons with * show p-values that survive Bonferroni correction at $P<0.05$.

	Network	Quade's ANOVA	Mann-Whitney U		
			Patients vs. Relative	Patients vs. Controls.	Relatives vs. Controls
Bonferroni corrected and adjusted for age and level of vigilance	GSW	0.105	1.000	0.102	0.225
	Sensorimotor	0.002*	1.000	0.024*	0.006*
	Occipital	0.776	1.000	1.000	1.000

Table 1.

P-values for group comparisons of synchrony in each of the three canonical networks. We report here p-values with Bonferroni correction and adjustment for age and level of vigilance.

Note that: * denotes significant ($P<0.05$)

Appendix

GGE patients					Unaffected GGE relatives			Healthy controls	
Age	**Gender**	**Syndrome**	**PS**	**AEDs**	**Age**	**Gender**	**Syndrome**	**Age**	**Gender**
16	Female	GTCSO	Y	None	22	Female	GTCSO	17	Female
17	Female	GTCSO	N	None	24	Female	GTCSO	20	Female
39	Male	GTCSO	N	LEV	42	Female	GTCSO	20	Female
13	Female	JME	Y	None	46	Female	GTCSO	22	Female
15	Female	JME	N	None	52	Female	GTCSO	23	Female
16	Female	JME	Y	None	25	Male	GTCSO	23	Female
20	Female	JME	N	None	33	Male	GTCSO	25	Female
22	Female	JME	N	LMT, LEV	51	Male	GTCSO	25	Female
22	Female	JME	N	LMT	22	Female	JME	28	Female
26	Female	JME	N	None	27	Female	JME	21	Male
20	Male	JME	--	None	37	Female	JME	22	Male
20	Male	JME	Y	VPA, LEV	51	Female	JME	23	Male
21	Male	JME	Y	VPA	55	Female	JME	24	Male
					58	Female	JME	24	Male
					18	Male	JME	24	Male
					52	Male	JME	26	Male
					56	Male	JME	29	Male
								34	Male

Table A1

Demographic characteristics of the three subject groups. Abbreviations: GGE, Genetic Generalised Epilepsy; JME, Juvenile Myoclonic Epilepsy; GTCSO, Generalized Tonic-Clonic Seizures Only (GCTSO); PS, Photosensitivity; Y, Yes; N, No; --, data is not available; LEV, Levetiracetam; LMT, Lamotrigine; VPA, Valproic acid

GSW	**Sensorimotor**	**Occipital**
'Precentral_L'	'Precentral_L'	'Calcarine_L'
'Precentral_R'	'Precentral_R'	'Calcarine_R'
'Frontal_Sup_L'	'Supp_Motor_Area_L'	'Cuneus_L'
'Frontal_Sup_R'	'Supp_Motor_Area_R'	'Cuneus_R'
'Frontal_Mid_L'	'Cingulum_Mid_L'	'Lingual_L'
'Frontal_Mid_R'	'Cingulum_Mid_R'	'Lingual_R'
'Frontal_Inf_Tri_L'	'Postcentral_L'	'Occipital_Sup_L'
'Frontal_Inf_Tri_R'	'Postcentral_R'	'Occipital_Sup_R'
'Supp_Motor_Area_L'		'Occipital_Mid_L'
'Frontal_Sup_Medial_L'		'Occipital_Mid_R'
'Frontal_Sup_Medial_R'		'Occipital_Inf_L'
'Cingulum_Mid_L'		'Occipital_Inf_R'
'Cingulum_Mid_R'		'Fusiform_R'
'Precuneus_L'		
'Precuneus_R'		

Table A2

Three canonical networks (GSW, sensorimotor and occipital networks) and their nodes. Abbreviations: L/R, left/right; Sup, superior; Mid, middle; Inf, Inferior; Supp, supplemental[1].

Reference

1. Tangwiriyasakul C, Perani S, Centeno M, et al. Dynamic brain network states in human generalized spike-wave discharges. *Brain* (October 2018).

Network	Patients			Relatives	
	By Sex	By Syndrome	By Photosensitivity	By Sex	By Syndrome
GSW	0.64	0.24	0.08	0.76	0.44
Sensorimotor	1.00	0.40	0.81	0.92	0.50
Occipital	0.44	0.61	0.29	0.92	0.44

Table A3

P-values from Mann-Whitney U test for group comparisons in each of the three canonical network. We report here p-values with age and level of vigilance.

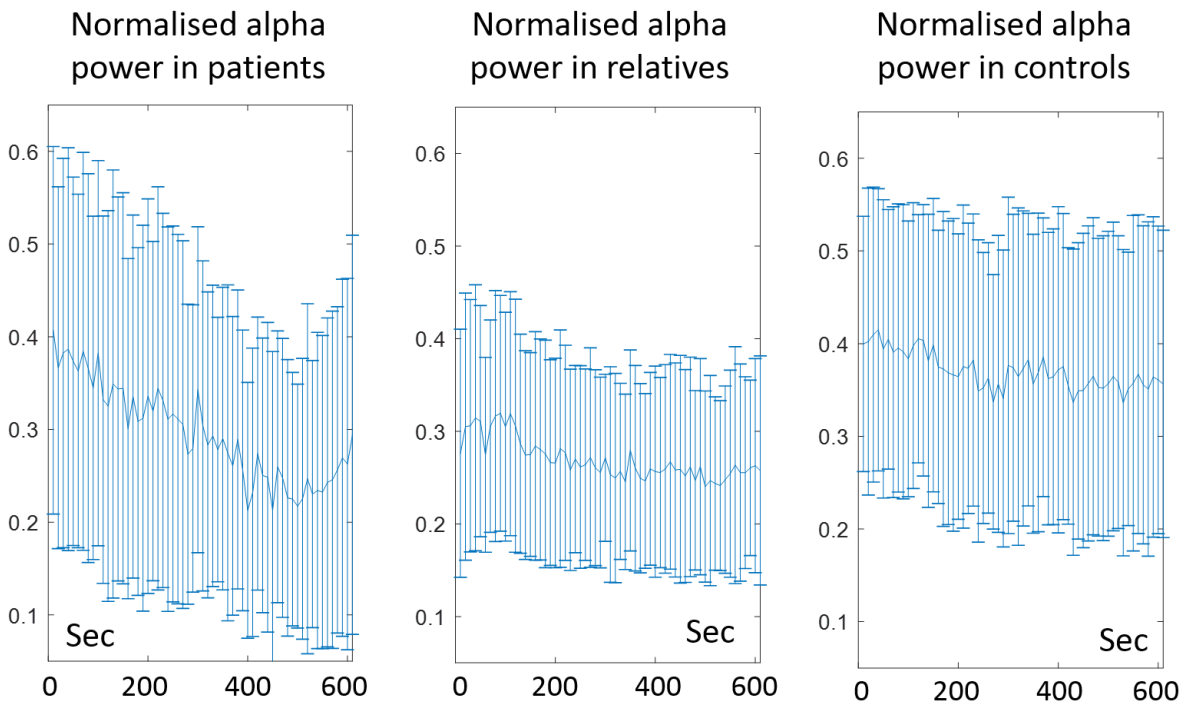

Figure A1: Evolution of averaged normalised alpha power and its standard deviation estimated across O1, O2, and Oz (from left to right: patients, 1st-degree relatives, and controls) over the course of time. Note that: we found no significant difference among the level of vigilance between the groups (slope of normalise alpha).